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(71) Applicant (for all designated States except US): GLAXO
GROUP LIMITED [GB/GB]; Glaxo Wellcome House,
Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SIMS, Martin, John
[GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Steve-
nage, Hertfordshire SG1 2NY (GB). HAYES, David, John
[GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Steve-
nage, Hertfordshire, SG1 2NY (GB).

(74) Agent: CRAWLEY, Karen; GlaxoSmithKline, Corporate
Intellectual Property (CN9.25.1), 980 Great West Road,
Brentford, Middlesex TW8 9GS (GB).

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(54) Title: ASSAY

WO (57) Abstract: This invention relates to truncated Tec kinase polypeptides and their use in screening for compounds which modulate the activity of Tec kinase polypeptides. Also described are nucleotide sequences encoding truncated Tec kinase polypeptides, vectors and host cells containing said nucleotides.

Assay**Field of invention**

5 The present invention relates to truncated Tec kinase polypeptides, nucleotide sequences encoding truncated Tec kinase polypeptides, vectors and host cells containing said nucleotides, methods of screening for compounds which modulate the activity of Tec kinase polypeptides, and the use of compounds identifiable by said method in therapy.

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Background to the invention

Antigen receptors on T, B and mast cells are multimolecular complexes that are activated by interactions with external signals. These signals are then transmitted to regulate gene expression and posttranscriptional modifications. A 15 family of non-receptor tyrosine kinases known as the "Tec kinase family", including Itk, Tec, Btk, Bmx and Txk tyrosine kinases, is involved in the signal transduction in T, B and mast cells. The members of the Tec kinase family share a similar domain structure, having an N-terminal pleckstrin-homology (PH) 20 domain; a Tec homology domain (TH), which includes one (Itk, Bmx, Txk, and Tec29) or two (Btk and Tec) proline-rich regions (PR); Src homology 3 (SH3) and Src homology 2 (SH2) domains; and a catalytic kinase domain (SH1), see for example Yang *et al.* (2000) Immunity 12:373-382.

25 A need exists to identify modulators of Tec kinase polypeptides in order to provide compounds for use in therapy. At present, there are no methods available for screening Tec kinase polypeptides. Accordingly, the object of the present invention is to provide a screen for compounds which modulate the activity of Tec kinase polypeptides and thereby provide compounds for use in 30 therapy.

Summary of Invention

The present invention is based on the finding that a truncated Tec kinase polypeptide can be used in an assay to screen for compounds which modulate the activity of Tec kinase polypeptides.

Accordingly, the invention provides a truncated Tec kinase polypeptide having a Tec kinase amino acid sequence truncated by a minimum of the amino acids constituting the PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the kinase domain.

Another aspect of the invention is an isolated polynucleotide which (a) encodes a truncated Tec kinase polypeptide of the invention; (b) is complementary to polynucleotide (a); (c) selectively hybridises to polynucleotide (a) or (b); or (d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

Further aspects of the invention are:

- an expression vector which comprises a polynucleotide of the invention and which is capable of expressing a polypeptide of the invention;
- a host cell comprising an expression vector of the invention;
- a primer which is capable of generating a polynucleotide of the invention;
- a method of producing a polypeptide of the invention which method comprises introducing into an appropriate cell line a vector comprising a polynucleotide of the invention under conditions suitable for obtaining expression of the polypeptide;
- a method for the identification of a compound which modulates the activity of a Tec kinase polypeptide, comprising contacting a polypeptide of the invention with a test compound and detecting any enhancement or inhibition in the activity

of the polypeptide, compared to the activity that would occur in the absence of said test compound;

- a compound which modulates Tec kinase activity and is identifiable by the method referred to above;

5 - use of a compound which modulates Tec kinase activity and is identifiable by the method referred to above for the manufacture of a medicament for the treatment or prophylaxis of a disorder that is responsive to modulation of Tec kinase activity; and

10 - a method of treating a subject having a disorder that is responsive to modulation of Tec kinase activity, which method comprises administering to said subject an effective amount of a compound identifiable by the method referred to above.

Detailed Description of the Invention

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We have surprisingly found that a truncated Tec kinase polypeptide, which is truncated by a minimum of the amino acids constituting the PH domain and at least one of the proline rich region of the TH domain, up to but not including the amino acids constituting the kinase domain, is suitable for screening for compounds which modulate the activity of Tec kinase polypeptides. The truncated Tec kinase polypeptides of the present invention are constitutively active despite truncation of a large portion of the protein.

25 The polypeptides of the invention have been found to be particularly suitable for screening as they do not need to be pre-activated by phosphorylation. *In vivo*, Tec kinases need to be phosphorylated by other kinases in order to activate the enzyme (Gibson *et al.* (1996) J. Immunology 156:2716-2722). Whilst pre-activation by phosphorylation is commonly required in assays, for example, LCK assays include a phosphorylation step (Trevillyan, *et al* (1999) Arch. Biochem. 30 Biophys. 364, 19-29), the removal of the need to preactivate the polypeptides of the present invention offers a simplification for the assay.

A further advantage of the present invention is the provision of an assay that is "robust". The present inventors have found that the assay is robust in two respects. Firstly, it is possible to generate large amounts of truncated enzyme which are stable over a long time. Secondly, the assay gives a high frequency of comparable results upon repeat testing.

The polypeptides of the invention may therefore provide useful screening targets for the identification and development of novel pharmaceutical agents, including agonists and antagonists of Tec kinases, which may be useful in the treatment and/or prophylaxis of disorders such as inflammation.

Truncated Tec Kinase Polypeptides

The invention provides a truncated Tec kinase polypeptide having a Tec kinase amino acid sequence truncated by a minimum of the amino acids constituting the PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the kinase domain. In other words, the point of truncation can occur anywhere after the first proline rich region of the TH domain (i.e. the proline rich region closest to the PH domain) and before the kinase domain. Preferably, the Tec kinase amino acid sequence is truncated by a minimum of the amino acids constituting the PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the SH2 and kinase domain, i.e. the point of truncation can occur anywhere after the first proline rich region of the TH domain and before the SH2 domain. More preferably, the Tec kinase amino acid sequence is truncated by the amino acids constituting PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the SH3, SH2 and kinase domain, i.e. the point of truncation can occur anywhere after the first proline rich region of the TH domain and before the SH3 domain. In a preferred aspect of the invention, when the Tec

kinase polypeptide contains more than one proline rich region, the truncated Tec kinase polypeptide does not contain any proline rich regions, i.e. the point of truncation occurs after all the proline rich regions of the TH domain.

5 The domains of the Tec kinase polypeptides referred to above are defined in Figures 5 and 6. Figure 6 shows a schematic representation of the domain structure of Tec kinases.

10 The "PH domain" as described herein is the N-terminal domain of the Tec kinase polypeptide. It comprises β sheet structure.

The "TH domain" as described herein is the domain situated between the PH and SH3 domains of the Tec kinase polypeptide. It comprises a globular core and either one (Itk, Bmx, Txk, Tec29) or two (Tec, Btk) proline rich (PR) regions.

15 The "proline rich regions" as described herein are the proline rich regions in the TH domain. Preferably, a proline rich region comprises at least two proline residues within six consecutive amino acids, preferably at least three proline residues within six consecutive amino acids, more preferably at least four proline residues within six consecutive amino acids.

20 The "SH3 domain" as described herein is the domain situated between the TH and SH2 domains of the Tec kinase polypeptide. It comprises two β sheets positioned at approximately right angles to each other.

25 The "SH2 domain" as described herein is the domain situated between the SH3 and kinase domain of the Tec kinase polypeptide.

30 The "kinase domain" as described herein is the C-terminal domain of the Tec kinase polypeptide. The "kinase domain" may alternatively be known as the "SH1" domain.

A "Tec kinase polypeptide" as described herein is a polypeptide of the Tec kinase family, including Itk, Tec, Btk, Bmx and Tsk tyrosine kinases.

5 Figure 5 shows an alignment of the polypeptide sequences of Tec family kinases showing the PH, TH, SH3 and SH2 domains. The ClustalX analysis program was used to align the sequences (Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 24:4876-4882). The default settings were used.
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Accordingly, in a preferred aspect of the invention, the Tec kinase polypeptide is truncated at a position between amino acid 199 (last amino acid of the first proline rich region of the TH domain, when the sequence is aligned as shown in
15 Figure 5) and amino acid 451 (first amino acid of the kinase domain, when the sequence is aligned as shown in Figure 5). More preferably the Tec kinase polypeptide is truncated at a position between amino acid 199 (last amino acid of the first proline rich region of the TH domain, when the sequence is aligned as shown in Figure 5) and amino acid 417 (last amino acid of the SH2 domain,
20 when the sequence is aligned as shown in Figure 5). More preferably the Tec kinase polypeptide is truncated at a position between amino acid 199 (last amino acid of the first proline rich region of the TH domain, when the sequence is aligned as shown in Figure 5) and amino acid 333 (first amino acid of the SH2 domain, when the sequence is aligned as shown in Figure 5). Even more
25 preferably the Tec kinase polypeptide is truncated at a position between amino acid 199 (last amino acid of the first proline rich region of the TH domain, when the sequence is aligned as shown in Figure 5) and amino acid 224 (first amino acid of the SH3 domain, when the sequence is aligned as shown in Figure 5).

30 In a more preferred aspect of the invention, when the Tec kinase polypeptide has more than one proline rich region, the Tec kinase polypeptide is truncated at

a position between amino acid 213 (last amino acid of the second proline rich region of the TH domain, when the sequence is aligned as shown in Figure 5) and amino acid 451 (first amino acid of the kinase domain, when the sequence is aligned as shown in Figure 5). More preferably the Tec kinase polypeptide is 5 truncated at a position between amino acid 213 (last amino acid of the second proline rich region of the TH domain, when the sequence is aligned as shown in Figure 5) and amino acid 417 (last amino acid of the SH2 domain, when the sequence is aligned as shown in Figure 5). More preferably the Tec kinase polypeptide is truncated at a position between amino acid 213 (last amino acid of 10 the second proline rich region of the TH domain, when the sequence is aligned as shown in Figure 5) and amino acid 333 (first amino acid of the SH2 domain, when the sequence is aligned as shown in Figure 5). Even more preferably the Tec kinase polypeptide is truncated at a position between amino acid 213 (last amino acid of the second proline rich region of the TH domain, when the sequence is aligned as 15 shown in Figure 5) and amino acid 224 (first amino acid of the SH3 domain, when the sequence is aligned as shown in Figure 5).

In a particular aspect of the invention, the start of the SH3 domain is defined by 20 the amino acid sequence: *X-X-V-[VIK]-A-[LM]-Y-D-[YF]; the start of the SH2 domain is defined by the amino acid sequence: [IL]-[ED]-X-Y-E-*W-Y; and the start of the kinase domain is defined by the amino acid sequence: G-[LF]-[GRS]-Y-[GDE]-[SK]-W-*¹, where,

- the letters denote amino acids in one letter code,
- the square brackets denote a single amino acid,
- the amino acids within the square brackets are alternatives,
- X is any one amino acid residue, and
- "*" indicates the start of the domain.

For example, the Tec kinase polypeptide sequences to be truncated may be 30 selected from Btk (human) – Accession no. Q06187 (SwissProt), Btk (mouse) - Accession no. P35991 (SwissProt), Itk (human) - Accession no. Q08881

(SwissProt), Itk (mouse) - Accession no. Q03526 (SwissProt), Tec (human) - Accession no. P42680 (SwissProt), Tec (mouse) - Accession no. P24604 (SwissProt), Bmx (human) - Accession no. P51813 (SwissProt), Bmx (mouse) - Accession no. P97504 (TREMBL), Txk (human) – Accession no. P42681 (SwissProt), and Txk (mouse) - Accession no. P42682 (SwissProt).

Preferably the truncated Tec kinase polypeptide is a truncated Itk polypeptide or a truncated Btk polypeptide. Preferably the Itk polypeptide to be truncated has a sequence as set forth in Figure 4 ("ITK" sequence) or a homolog or variant thereof. Preferably, the truncated Itk polypeptide has the polypeptide sequence set forth in Figure 3 or a homolog or variant thereof. More preferably, the truncated Itk polypeptide has a sequence encoded by the polynucleotide sequence set forth in Figure 2. Preferably the Btk polypeptide to be truncated has a sequence Btk (human) Accession no. Q06187 (Swiss prot. Database) or a homolog or variant thereof. Preferably, the truncated Btk polypeptide has the polypeptide sequence set forth in Figure 8 or a homolog or variant thereof. More preferably, the truncated Btk polypeptide has a sequence encoded by the polynucleotide sequence set forth in Figure 9.

The polypeptides of the present invention are provided in an isolated form. The term "isolated" is intended to convey that the material is not in its native state. Thus, the naturally-occurring polypeptide present in a living animal is in its native state and is not isolated, but the same polypeptide, separated from some or all of the materials it co-exists with in the natural system, is isolated. The polypeptides may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as isolated. Similarly, a polypeptide which has been produced by synthetic means, for example, by recombinant methods is "isolated". The polypeptides of the present invention are also preferably provided in purified form, and preferably are purified to at least 50% purity, more preferably about 75% purity, most preferably 90% purity or greater, such as 95%, 98% pure. Routine methods can be employed to purify and/or

5 synthesize the polypeptides according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook *et al*, Molecular Cloning: a Laboratory Manual, 2nd Edition, CSH Laboratory Press, 1989, the disclosure of which is included herein in its entirety by way of reference.

10 The polypeptide of the present invention may be a recombinant or a synthetic polypeptide. The polypeptide of the invention is a human or animal sequence (or homologous to such sequence). Such an animal is typically a mammal, such as a rodent (e.g. a mouse) or a primate. Preferably the polypeptide is a human sequence.

15 Homologues of polypeptide sequences are referred to above. Such homologues typically have at least 70% homology, preferably at least 80%, 90%, 95%, 97% or 99% homology, for example over a region of at least 15, 20, 30, 100 or more contiguous amino acids. The homology may be calculated on the basis of amino acid identity (sometime referred to as "hard homology"). Identity is calculated using the widely used GCG (University of Wisconsin) suite of programs and preferably using the distances software (correction method).

20 The term "variant" refers to a polypeptide which has a same essential character or basic biological functionality as the truncated Tec kinase polypeptide in question. Preferably a variant polypeptide is one which binds to the same ligand as the truncated Tec kinase polypeptide. Such variants may include allelic variants and the deletion, modification or addition of single amino acids or groups of amino acids within the polypeptide sequence.

25 Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 15, 20 or 30 substitutions. The modified polypeptide retains activity as a truncated Tec kinase polypeptide. Changes in amino acid sequence of peptides can be guided by known similarities among amino acids and other molecules or substituents in

physical features such as charge density, hydrophobicity, hydrophilicity, size and configuration etc. For example, the amino acid Thr may be replaced by Ser and vice versa, and Leu may be replaced by Ile and vice versa. For example, a polar amino acid such as glycine or serine may be substituted for another polar amino 5 acid; a basic amino acid may be substituted for another basic amino acid; an acidic amino acid may be substituted for another acidic amino acid; or a non-polar amino acid may be substituted for another non-polar amino acid. Groups of amino acids normally considered to be equivalent are:

- 10 (a) Ala (A), Ser (S), Thr (T), Pro (P), Gly (G);
(b) Asn (N), Asp (D), Glu (E), Gln (Q);
(c) His (H), Arg (R) Glu (E), Gln (Q);
(d) Met (M), Leu (L), Ile (I), Val (V); and
(e) Phe (F), Tyr (Y), Trp (W).

15 Polypeptides of the invention may be chemically modified, e.g. post-translationally modified. For example, they may be glycosylated or comprise modified amino acid residues. They may also be modified by the addition of histidine residues to assist their purification, or other suitable protein tags, see 20 for example, Nilsson *et al.* (1997) Protein Expression and Purification 11:1-16. Such modified polypeptides fall within the scope of the term "polypeptide" of the invention.

Polynucleotides and primers

25 A further aspect of the invention is an isolated polynucleotide which (a) encodes a truncated Tec kinase polypeptide of the invention; (b) is complementary to polynucleotide (a); (c) selectively hybridises to polynucleotide (a) or (b); or (d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c). A 30 preferred aspect is an isolated polynucleotide which: (a) encodes the truncated Itk polypeptide set forth in Figure 3; (b) is complementary to polynucleotide (a);

(c) selectively hybridises to polynucleotide (a) or (b); or (d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c). A more preferred aspect of the invention is an isolated polynucleotide having (a) the sequence set forth in Figure 2; (b) a sequence complementary to polynucleotide (a); (c) a sequence which selectively hybridises to polynucleotide (a) or (b); or (d) a sequence that is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c). A further aspect of the invention, is an isolated polynucleotide which: (a) encodes the truncated Btk polypeptide set forth in Figure 8; (b) is complementary to polynucleotide (a); (c) selectively hybridises to polynucleotide (a) or (b); or (d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c). A more preferred aspect of the invention is an isolated polynucleotide having (a) the sequence set forth in Figure 9; (b) a sequence complementary to polynucleotide (a); (c) a sequence which selectively hybridises to polynucleotide (a) or (b); or (d) a sequence that is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

The polynucleotide sequences of the present invention may be in the form of RNA or in the form of DNA, for example cDNA, genomic DNA, and synthetic DNA. Preferably the polynucleotide sequence of the invention is cDNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand.

As used herein the term polynucleotide includes nucleic acids that contain one or more modified (e.g., tritylated) or unusual (e.g., inosine) bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are polynucleotides as that term is intended herein.

The polynucleotide of the invention is a human or animal sequence (or homologous to such sequence). Such an animal is typically a mammal, such as a rodent (e.g. a mouse) or a primate. Preferably the polynucleotide is a human sequence.

Homologues of polynucleotide sequences typically have at least 70% sequence identity, preferably at least 80%, 90%, 95%, 97% or 99% sequence identity, for example over a region of at least 15, 20, 30, 100 or more contiguous nucleotides. Methods of measuring nucleic acid homology are well known in the art. For example, the UWGCG Package provides the BESTFIT program which can be used to calculate homology (Devereux *et al* 1984). Similarly the PILEUP and BLAST algorithms can be used to line up sequences (for example are described in Altschul 1993, and Altschul *et al* 1990). In accordance with the invention, the default settings may be used.

As used herein, "selective hybridisation" means that generally the polynucleotide can hybridize to the gene region sequence at a level significantly above background. The signal level generated by the interaction between a polynucleotide of the invention and the gene region sequence is typically at least 10 fold, preferably at least 100 fold, as intense as interactions between other polynucleotides and the gene region sequence. The intensity of interaction may be measured, for example, by radiolabelling the polynucleotide, e.g. with ^{32}P . Selective hybridisation may typically be achieved using conditions of low stringency (0.3M sodium chloride and 0.03M sodium citrate at about 40°C), medium stringency (for example, 0.3M sodium chloride and 0.03M sodium citrate at about 50°C) or high stringency (for example, 0.03M sodium chloride and 0.003M sodium citrate at about 60°C).

The coding sequence of polynucleotides of the present invention may be modified by nucleotide substitutions, for example from 1, 2 or 3 to 10, 15, 25, 50 or 100 substitutions. The polynucleotide may alternatively or additionally be modified by one or more insertions and/or deletions and/or by an extension at either or both ends. The modified polynucleotide encodes a polypeptide which has the activity associated with a truncated Tec kinase polypeptide. Degenerate substitutions may be made and/or substitutions may be made which would result

in a conservative amino acid substitution when the modified sequence is translated, for example as shown above.

Generation of a Tec kinase oligonucleotide may be performed by methodologies known in the art such as polymerase chain reaction (PCR) for example on genomic DNA or cDNA with appropriate oligonucleotide primers derived from or designed based on a knowledge of the sequence of the Tec kinase of interest. For example, the polynucleotide sequences of Tec kinases may be selected from: Btk (human) - Accession no. X58957 (EMBL/GenBank), Btk (mouse) - Accession no. L08967 (EMBL/GenBank), Itk (human) - Accession no. D13720 (EMBL/GenBank), Itk (mouse) - Accession no. L00619 (EMBL/GenBank), Tec (human) - Accession no. D29767 (EMBL/GenBank), Tec (mouse) - Accession no. S53716 (EMBL/GenBank), Bmx (human) - Accession no. X83107 (EMBL/GenBank), Bmx (mouse) - Accession no. U88091 (EMBL/GenBank), Txk (human) - Accession no. L27071 (EMBL/GenBank), and Txk (mouse) - Accession no. U16145 (EMBL/GenBank).

According to a further aspect of the invention, there is provided a primer which is capable of generating a Tec kinase polynucleotide which encodes a truncated Tec kinase polypeptide of the invention. An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides discrimination between alleles through selective amplification of one allele at a particular sequence position. The allele specific primer is preferably at least 10, preferably at least 15 or at least 20, for example at least 25, at least 30 nucleotides in length. For example, in the generation of a truncated Itk polypeptide, the primers shown in Figure 1 may be used to generate the corresponding polynucleotide. In the generation of a truncated Btk polypeptide, the primers shown in Figure 7 may be used to generate the corresponding polynucleotide.

The polynucleotide sequences of the present invention are provided in an isolated form. The term "isolated" is intended to convey that the material is not in its native state. Thus, the naturally-occurring polynucleotide sequence present in a living animal is in its native state and is not isolated, but the same 5 polynucleotide sequence, separated from some or all of the materials it co-exists with in the natural system, is isolated. They may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as isolated. Such polynucleotide sequence could be part of a vector. Such polynucleotide sequence could be part of a composition, and still be isolated in 10 that such vector or composition is not part of its natural environment. The polynucleotide sequences of the present invention are also preferably provided in purified form, and preferably are purified to at least 50% purity, more preferably about 75% purity, most preferably 90% purity or greater, such as 95%, 98% pure.

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Production of Tec kinase polypeptides

The polynucleotide sequences of the present invention may be employed for producing a polypeptide of the invention by recombinant techniques. Thus, for 20 example the nucleotide sequence may be included in any one of a variety of expression vehicles or cloning vehicles, in particular vectors or plasmids for expressing a protein.

A further aspect of the invention is therefore a vector comprising a 25 polynucleotide of the invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts include mammalian expression vectors, insect expression vectors, yeast expression vectors, bacterial expression vectors and viral expression vectors, see Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY., (1989). Examples of suitable vectors include derivatives of bacterial plasmids; phage 30

DNA; yeast plasmids; vectors derived from combinations of plasmids and phage DNA and viral DNA and baculoviruses. A preferred vector is a baculovirus.

5 In a preferred aspect, the vector further comprises one or more regulatory sequences to direct mRNA synthesis, including, for example, a promoter, operably linked to the sequence. Suitable promoters include: insect cell promotor, polyhedrin promotor, p10 and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The vector may contain an enhancer and a ribosome binding site for translation initiation
10 and a transcription terminator. Large numbers of suitable vectors and promoters/enhancers, will be known to those of skill in the art, but any plasmid or vector, promoter/enhancer may be used as long as it is replicable and functional in the host. The vector may also include appropriate sequences for selection and/or amplification of expression. For this the vector will comprise one or more
15 phenotypic selectable/amplifiable markers. Such markers are also well known to those skilled in the art.

In a further aspect, the present invention provides host cells comprising a vector of the invention, and capable of expressing a nucleotide sequence of the invention. The host cells can be, for example, a higher eukaryotic cell, such as a mammalian cell or a lower eukaryotic cell, such as a yeast cell or a prokaryotic cell such as a bacterial cell. Suitable prokaryotic hosts for transformation include E-coli. Suitable eukaryotic hosts include insect cells, e.g. SF9 cells, Tni cells, Hi5 cells, and mammalian cells, e.g. HEK HeLa, COS, CHO, NSO 3T3 (fibroblast cell line). Cells expressing a nucleotide sequence of the invention can be lysed to obtain the polypeptide of the invention, which may optionally be purified.
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Cell free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

A further aspect of the invention is a method of producing a polypeptide of the invention, which method comprises introducing into an appropriate cell line a vector comprising a polynucleotide as defined herein under conditions suitable for obtaining expression of the polypeptide.

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Methods of screening

The present invention further provides a method for identification of a compound which modulates Tec kinase activity. Compounds which modulate Tec kinase activity are those which inhibit or enhance the function of the Tec kinase polypeptides (e.g. to act as antagonists or agonists of the Tec kinase protein function). In general terms, the screen for such compounds will comprise contacting a polypeptide of the invention with a test compound, and then detecting any enhancement or inhibition of polypeptide activity that results (compared to the activity that would occur in the absence of the test compound). By contacting, it is meant that the test compound and the polypeptide of the invention are in such proximity that they are able to interact biologically. The polypeptides of the invention may be used in high throughput screens, thus enabling large numbers of compounds to be studied.

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The activity that is detected is preferably phospho-transfer activity. Phospho-transfer involves the transfer of a phosphate from ATP to a tyrosine residue contained within the substrate. Phospho-transfer activity may be detected by the inclusion of a target polypeptide in the assay. More particularly, polypeptides of the invention may be used in any suitable tyrosine kinase assay, for example, by a time-resolved fluorescence assay, such as homogenous time-resolved fluorescence (HTRF®, Packard Instrument Company) Kolb et al., DDT Vol. 3, 333-342; in-plate binding assays including colorimetric and luminescent read-outs and time-resolved fluorescence, see for example Farley et al., Anal. Biochem (1992) 203, 151-157, Lehel et al., (1997) Anal. Biochem. 244, 340-346 and Braunwalder et al., (1996) Anal. Biochem. 238, 340-346; or radiometric

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assays, for example, using ^{33}P or ^{32}P , such as scintillation proximity assay (SPA, Amersham International). Examples of time-resolved fluorescence assays are: in-plate time-resolved fluorescence (trf) assay (Delfia®, Wallac Oy). Preferably a time-resolved fluorescence assay is used, most preferably homogenous time-resolved fluorescence.

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In a preferred aspect, the method of screening is carried out using cells expressing a polypeptide of the invention, and incubating such cells with the test compound, optionally in the presence of a Tec kinase ligand.

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Methods of treatment

A further aspect of the present invention is a compound which modulates Tec kinase activity and is identifiable by screening techniques referred to above. Preferably, the compound has been identified using the above screening techniques. The compounds may be agonists or antagonists of the Tec kinase polypeptide, but preferably are antagonists. The compounds include, for example, aptamers, polypeptides, antibodies and small molecules. The compounds may be useful in the treatment and/or prophylaxis of disorders that are responsive to modulation of Tec kinase activity. For example, it has been demonstrated *in vivo* that Itk-deficient mice are unable to establish TH2 cells suggesting that Itk has a role in mediating the development of IL-4 producing TH2 cells (Fowell *et al.* (1999) *Immunity* 11:399-409). The absence of this cytokine in Itk-deficient mice suggests that compounds which modulate Tec kinases may be useful in the treatment of inflammatory diseases such as, for example, asthma.

One particular aspect of the invention is the use of a compound which modulates Tec kinase activity and is identifiable by the screening techniques of the invention, for the manufacture of a medicament for the treatment or prophylaxis

of disorders that are responsive to modulation of the activity of Tec kinase activity, such as inflammation.

Another aspect of the invention is a method of treating a subject having a disorder which is responsive to modulation of Tec kinase activity, such as inflammation, which method comprises administering to a subject an effective amount of a compound identifiable by the screening techniques of the invention.

Examples of inflammatory conditions include: asthma, allergic rhinitis, URID (upper respiratory inflammatory disease), adult respiratory distress syndrome; arthritic conditions such as rheumatoid arthritis, rheumatoid spondylitis, and osteoarthritis; inflammatory eye conditions such as uveitis (including iritis) and conjunctivitis; inflammatory bowel conditions such as Crohn's disease, ulcerative colitis and distal proctitis; periodontal disease; esophagitis, inflammatory skin conditions such as psoriasis, eczema and dermatitis. Preferred inflammatory conditions are asthma, allergic rhinitis and URID (upper respiratory inflammatory disease).

Where the Tec kinase is Btk, preferably the disorder is selected from inflammatory conditions (described above); diseases with a B cell component such as B cell leukaemias and SLE (Systemic Lupus erythematosis); and diseases associated with platelet function.

Substances identified according to the screening methods outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art. The exact nature of a formulation will depend upon several factors including the particular substance to be administered and the desired route of administration. The substances may be administered by enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intra-arterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

5

Therapeutically effective amounts of such compounds can be readily determined by those skilled in the art using, e.g. dose-response studies. The dose of agent may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration and the required regime. A suitable dose may however be from 0.01 to 50mg/kg body weight such as 1 to 40 mg/kg body weight. A physician will be able to determine the required route of administration and dosage for any particular patient.

10

Brief Description of the Figures:

15 **Figure 1** shows the oligonucleotide sequences used to generate the truncated Itk construct shown in Figure 2.

Figure 2 shows the polynucleotide sequence of the truncated Itk construct (including cloning sites, underlined).

20 **Figure 3** shows the polynucleotide and translated polypeptide sequence of the truncated Itk construct.

25 **Figure 4** shows an alignment of the polypeptide sequences of Itk (as in Swissprot data base accession number: Q08881) and the truncated construct showing PH; SH3; SH2 and kinase domains. The domain boundaries are as defined by Swissprot entry Q08881: PH 4-111; SH3 171-23; SH2 239-338; Kinase 363-615. Note: tITK = truncated ITK.

30 **Figure 5** shows an alignment of the polypeptide sequences of Tec family kinases (SwissProt database);

Btk (human) – Accession no. Q06187, Btk (mouse) - Accession no. P35991, Itk (human) - Accession no. Q08881, Itk (mouse) - Accession no. Q03526, Tec (human) - Accession no. P42680, Tec (mouse) - Accession no. P24604, Bmx (human) - Accession no. P51813, Txk (human) - P42681/Q14220, and Txk (mouse) - Accession no. P42682, showing the PH domain (Bold, italic and boxed), Tec homology domain (Italic and boxed), SH3 domain (Bold and boxed) and SH2 domain (Boxed).

Figure 6 shows a schematic representation of the domain structure of Tec kinases.

Figure 7 shows the oligonucleotide sequences used to generate the truncated Btk construct shown in Figure 9.

Figure 8 shows the translated polypeptide sequence of the truncated Btk construct.

Figure 9 shows the polynucleotide sequence of the truncated Btk construct.

Note: the full length sequence is available on the genembl database accession number X58957.

Examples

Example 1A: Generation of the truncated Itk construct

To generate an active form of Itk oligonucleotide, PCR primers were designed to amplify, from cDNA, a single region of Itk corresponding to the combined SH3, SH2 and kinase domains and to incorporate a start methionine and restriction endonuclease sites for cloning the construct. The Oligonucleotide sequences used to generate the truncated Itk construct are shown in Figure 1. A T cell cDNA library was used as a source of template DNA (generation of library

described in Biotechniques (1998) 25:85-92). A PCR product was cloned (Figure 2), sequenced and used to generate a recombinant baculovirus for infection of SF9 insect cells using standard molecular biological techniques (see for example, Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, 2nd Edition, CSH Laboratory Press, 1989).

5 **Example 1B: Protein expression and assay formation**

Insect cell pellets infected with the recombinant baculovirus described in
10 Example 1A were homogenised in 40 mM HEPES (pH 7.4), 100 mM NaCl 2 mM
EDTA, 10% glycerol, 0.1 mM vanadate and protease inhibitors. The 100 000 g
supernatant was stored at -85°C. Stored lysates were thawed on ice, ATP and
MgCl₂ (0.1 mM and 10 mM) were added. Following incubation on ice the kinase
15 was diluted in 40 mM HEPES (pH 7.4). The kinase reaction mixture contained
40 mM HEPES (pH 7.4), 10 mM MgCl₂, 0.05 mM ATP, 0.0005 mM peptide
(Biotin-AAAEEIYGEI). The reaction was stopped by the addition of EDTA (25
mM). The amount of phosphopeptide was quantitated by homogeneous time
resolved fluorescence as described in Kolb *et al.* (1998) Drug Discovery Today
15 3:333-342. An increase in the level of fluorescence indicates an increase in
kinase activity.
20

The Km for ATP and peptide was determined to be 0.039 +/- 0.011mM and
0.480 +/- 0.183 uM respectively.

25 **Example 1C: Screening compounds for modulation of Itk activity**

The table below shows inhibitors of Itk activity identified using the screen
described in Example 1B above.

30 **Compound testing in kinase assay**

Order of additions:

Compound to well

Enzyme to well

15 min pre-incubation

5 Substrates (ATP, src-peptide) added

30 min incubation

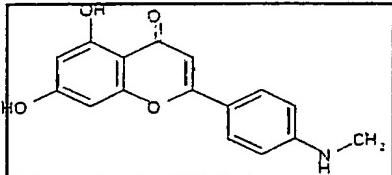
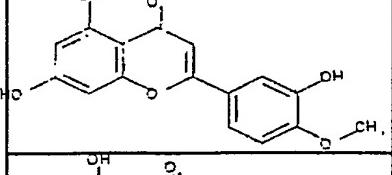
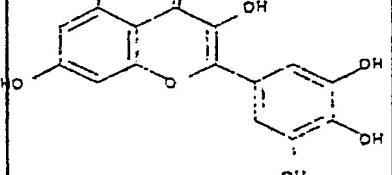
Reaction stopped with EDTA

HTRF reagents (APC, Eu labelled antibody (antiPY antibody)) added

Stand for 20 mins

10 Read signal on plate reader

UK: ITK

Class Name	Average PIC50	Average IC50 (uM)
	6.09	0.813
	6.09	0.813
	5.78	1.660

Note: PIC₅₀ is -log₁₀ IC₅₀ in molar, a higher PIC₅₀ indicates greater potency.

Example 1D: Performance of Itk Screen

5 The following data demonstrate the robustness of the Itk assay. Repetition of the assay described above over a period between 12/8/99 and 24/2/00 (Batch 1) and 22/3/00 and 25/5/00 (Batch 2) demonstrates: (i) it is possible to generate large amounts of truncated enzyme which are stable over a long time, and (ii) the assay gives a high frequency of comparable results upon repeat testing.

	Batch 1		Batch 2	
Date	12/8/99	24/2/00	22/3/00	25/5/00
Control	7076 ± 735	6373 ± 461	6978 ± 341	5616 ± 66
Blank	175 ± 7 31	193 ± 31	140 ± 15	120 ± 3
Signal/Noise	40	33	50	47

10 The results are expressed as the mean ± SD ($n \geq 80$).

Example 2A: Generation of the truncated Btk construct

15 To generate an active form of Btk oligonucleotide, PCR primers were designed to amplify, from cDNA, a single region of Btk corresponding to the combined SH3, SH2 and kinase domains and to incorporate a start methionine and restriction endonuclease sites for cloning the construct. The Oligonucleotide sequences used to generate the truncated Btk construct are shown in Figure 7.

20 A PCR product was cloned, sequenced and used to generate a recombinant baculovirus for infection of SF9 insect cells using standard molecular biological techniques (see for example, Sambrook *et al*, Molecular Cloning: a Laboratory Manual, 2nd Edition, CSH Laboratory Press, 1989).

Example 2B: Protein expression and assay formation

Insect cell pellets infected with the recombinant baculovirus described in Example 2A were homogenised in 40 mM HEPES (pH 7.4), 100 mM NaCl 2 mM EDTA, 10% glycerol, 0.1 mM vanadate and protease inhibitors. The 100 000 g supernatant was stored at -85°C. Stored lysates were thawed on ice and diluted in 40 mM HEPES (pH 7.4). The kinase reaction mixture contained 40mM HEPES pH7.4; 80mM MgCl₂; 1300μM ATP; 500nM peptide (Biotin-AAAEEIYGEI-NH₂). The reaction was stopped by the addition of EDTA (0.16 M). The amount of phosphopeptide was quantitated by homogeneous time resolved fluorescence as described in Kolb *et al.* (1998) Drug Discovery Today 3:333-342. An increase in the level of fluorescence indicates an increase in kinase activity.

The Km for ATP was determined to be 1.69 +/- 0.36mM

Example 2C: Screening compounds for modulation of Btk activity

The table below shows an inhibitor of Btk activity identified using the screen described in Example 2B above.

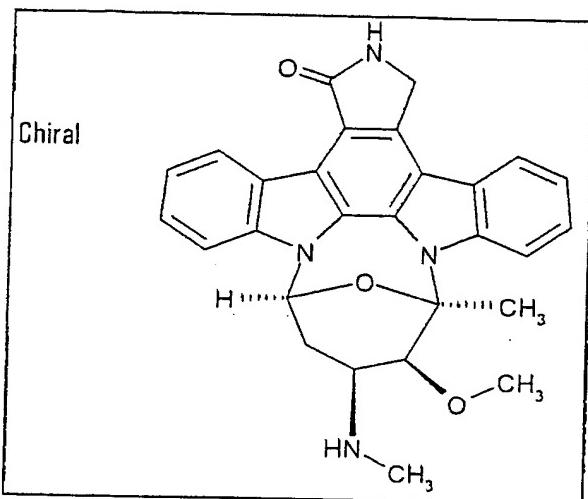
Compound testing in kinase assay

Order of additions:

Compound to well
Enzyme to well
15 min pre-incubation
Substrates (ATP, src-peptide) added
30 min incubation
Reaction stopped with EDTA
HTRF reagents (APC, Eu labelled antibody (antiPY antibody)) added
Stand for 20 mins

Read signal on plate reader

Compound: Staurosporin



$\text{pIC}_{50} = 6.83$

5

Note: pIC_{50} is $-\log_{10} \text{IC}_{50}$ in molar, a higher pIC_{50} indicates greater potency.

CLAIMS

1. A truncated Tec kinase polypeptide having a Tec kinase amino acid sequence truncated by a minimum of the amino acids constituting the PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the kinase domain.
5
2. A truncated Tec kinase polypeptide as claimed in claim 1 wherein the Tec kinase amino acid sequence is truncated by a minimum of the amino acids constituting the PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the SH2 and kinase domain.
10
3. A truncated Tec kinase polypeptide as claimed in claim 1 wherein the Tec kinase amino acid sequence is truncated by a minimum of the amino acids constituting the PH domain and a portion of the TH domain including at least one proline rich region up to but not including the amino acids constituting the SH3, SH2 and kinase domain.
15
4. A truncated Tec kinase polypeptide as claimed in any one of claims 1-3 which does not contain any proline rich regions.
20
5. A truncated Tec kinase polypeptide as claimed in any one of claims 1-4 wherein the Tec kinase polypeptide is Itk.
25
6. A truncated Tec kinase polypeptide as claimed in claim 5 having the amino acid sequence set forth in Figure 3 or at least 70% homology thereto.
7. A truncated Tec kinase polypeptide as claimed in any one of claims 1-4 wherein the Tec kinase polypeptide is Btk.
30

8. A truncated Tec kinase polypeptide as claimed in claim 7 having the amino acid sequence set forth in Figure 8 or at least 70% homology thereto.

9. An isolated polynucleotide which:

5 (a) encodes a truncated Tec kinase polypeptide as claimed in any one of claims 1-8;

(b) is complementary to polynucleotide (a);

(c) selectively hybridises to polynucleotide (a) or (b); or

10 (d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

10. An isolated polynucleotide as claimed in claim 9 which:

(a) encodes the truncated Itk polypeptide set forth in Figure 3;

(b) is complementary to polynucleotide (a);

15 (c) selectively hybridises to polynucleotide (a) or (b); or

(d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

11. An isolated polynucleotide as claimed in claim 10 having:

20 (a) the sequence set forth in Figure 2;

(b) a sequence complementary to polynucleotide (a);

(c) a sequence which selectively hybridises to polynucleotide (a) or (b); or

(d) a sequence that is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

25

12. An isolated polynucleotide as claimed in claim 9 which:

(a) encodes the truncated Btk polypeptide set forth in Figure 8;

(b) is complementary to polynucleotide (a);

(c) selectively hybridises to polynucleotide (a) or (b); or

30 (d) is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

13. An isolated polynucleotide as claimed in claim 12 having:

- (a) the sequence set forth in Figure 9;
- (b) a sequence complementary to polynucleotide (a);
- 5 (c) a sequence which selectively hybridises to polynucleotide (a) or (b); or
- (d) a sequence that is degenerate as a result of the genetic code from polynucleotide (a), (b) or (c).

14. A vector comprising a polynucleotide as claimed in any one of claims 9-13.

10

15. A host cell comprising a vector as claimed in claim 14.

16. A primer which is capable of generating a truncated Tec kinase polynucleotide as claimed in any one of claims 9-13.

15

17. A method of producing a polypeptide as claimed in any one of claims 1-8, which method comprises introducing into an appropriate cell line a vector comprising a polynucleotide as claimed in any one of claims 9-13 under conditions suitable for obtaining expression of the polypeptide.

20

18. A method for the identification of a compound which modulates the activity of a Tec kinase polypeptide, comprising contacting a polypeptide as claimed in any one of claims 1-8 with a test compound and detecting any enhancement or inhibition in the activity of the polypeptide, compared to the activity that would occur in the absence of said test compound.

25

19. A method for the identification of a compound as claimed in claim 18, comprising contacting a compound of interest with a polypeptide as claimed in any one of claims 1-8 in the presence of a target polypeptide and observing phosphorylation of the target polypeptide.

30

20. A method according to claims 18 or 19 wherein a cell line expressing a polypeptide as claimed in any one of claims 1-8 is contacted with the compound of interest.

5 21. A compound which modulates Tec kinase activity and is identifiable by the method according to any one of claims 18-20.

22. A compound identifiable by the method according to any one of claims 18-20 for use in therapy.

10 23. Use of a compound which modulates Tec kinase activity and is identifiable by the method of any one of claims 18-20 for the manufacture of a medicament for the treatment or prophylaxis of a disorder that is responsive to modulation of Tec kinase activity.

15 24. Use according to claim 23 wherein the disorder is inflammation.

20 25. A method of treating a subject having a disorder that is responsive to modulation of Tec kinase activity, which method comprises administering to a patient an effective amount of a compound identifiable by a method according to any one of claims 18-20.

26. The method according to claim 25 wherein the disorder is inflammation.

1 / 8

5 prime Oligo: gatcGGATCCATGCCTGAAGAAACTGTGGTC
 3 prime Oligo: gatcGGATCCCTAAAGTCCTGATTCTGC

FIG. 1

1	GGATCCATGCCTGAAGAAACTGTGGTCATTGCCTTATATGACTACCAACCAATGATCCT	60
61	CAGGAACTCGCACTGCAGCGAACGAAGACTGCCTGCTGGACAGTCTGAGATTAC	120
121	TGGTGGAGAGTCCAGGACAGGAATGGCATGAAGGATATGTACCAAGCAGTTATCTGGTG	180
181	GAAAAATCTCCAATAATCTGGAAACCTATGAGTGGTACAATAAGAGTATCAGCCGAGAC	240
241	AAAGCTGAAAAACTCTTTGGACACAGGAAAGAAGGAGCCCTCATGTAAGGGATTCC	300
301	AGGACTGCAGGAACATACACCGTGTCTGTTCACCAAGGCTGTTGTAAGTGAGAACAAAT	360
361	CCCTGTATAAACGATTATCACATCAAGGAAACAAATGACAATCCTAACGCGATACTATGTG	420
421	GCTGAAAAGTATGTGTTGATTCCATCCCTCTTCATCAACTATCACCAACATAATGGA	480
481	GGAGGCCTGGTGAUTCGACTCCGGTATCCAGTTGTTGGGAGGCAGAAAGCCCCAGTT	540
541	ACAGCAGGGCTGAGATACGGGAAATGGGTGATCGACCCCTCAGAGCTACTTTGTGCAA	600
601	GAGATTGGCAGTGGCAATTGGGTTGGTCATCTGGCTACTGGCTAACAAAGGACAAG	660
661	GTGGCTATCAAACCATCGGGAAAGGGCTATGTCAGAACAGAGGACTTCATAGAGGAGGCT	720
721	GAAGTAATGATGAAACTCTCTCATCCAAACTGGTGCAGCTGTATGGGGTGTGCCTGGAG	780
781	CAGGCCCATCTGCCTGGTTGAGTTCATGGAGCACGGCTGCCTGTCAGATTATCTA	840
841	CGCACCCAGCGGGACTTTGCTGCAGAGACCCCTGCTGGCATGTGTCAGGGATGTGT	900
901	GAGGCATGGCTACCTGGAAGAGGCATGTGTCATCCACAGAGACTTGGCTGCCAGAAAT	960
961	TGTTGGTGGAGAAACCAAGTCATCAAGGTGTCTGACTTGGGATGACAAGGTTCGTT	1020
1021	CTGGATGATCAGTACACCAGTTCCACAGGCACCAATTCCCGGTGAAGGGCATCCCCA	1080
1081	GAGGTTCTCTTCAGTCGCTATAGCAGCAAGTCCGATGTGGTCATTGGTGTGCTG	1140
1141	ATGTGGGAAGTTTCAGTGAAGGCAAAATCCGTATGAAAACCGAAGCAACTCAGAGGTG	1200
1201	GTGGAAGACATCAGTACCGGATTTCGGTTGTAAGCCCCGGCTGCCACACACGTC	1260
1261	TACCAAGATTATGAATCACTGCTGGAAAGAGAGACCAAGAGATCGGCCAGCCTCTCCAGA	1320
1321	CTGCTGCGTCAACTGGCTGAAATTGCAAGACTCAGGACTTTAGGGATCC	1368

FIG. 2

FIG. 3

1 GGATCCATGCCTGAAGAAACTGTGGTCATTGCTTATATGACTACCAACCAATGATCCT 60
 M P E E T V V I A L Y D Y Q T N D P
 61 CAGGAACCTCGCACTGCAGCGAACGAAGAGTACTGCCTGCTGGACAGTCTGAGATTAC 120
 Q E L A L R R N E E Y C L L D S S E I H
 121 TGGTGGAGAGTCCAGGACAGGAATGGGCATGAAGGATATGTACCAAGCAGTTATCTGGTG 180
 W W R V Q D R N G H E G Y V P S S Y L V
 181 GAAAAATCTCAAATAATCTGGAAACCTATGAGTGGTACAATAAGAGTATCAGCCGAGAC 240
 E K S P N N L E T Y E W Y N K S I S R D
 241 AAAGCTGAAAAACTCTTTGGACACAGGCAAAGAAGGAGCCTTCATGGTAAGGGATTCC 300
 K A E K L L D T G K E G A F M V R D S
 301 AGGACTGCAGGAACATACACCGTGTCTGTTTACCAAGGCTGTTGTAAGTGAGAACAAAT 360
 R T A G T Y T V S V F T K A V V S E N N
 361 CCCTGTATAAAGCATTATCACATCAAGGAAACAAATGACAATCCTAAGCGATACTATGTG 420
 P C I K H Y H I K E T N D N P K R Y Y V
 421 GCTGAAAAGTATGTGTTGACTCGACTCCGGTATCCAGTTGTTGGAGGCAGAAAGCCCCAGTT 480
 A E K Y V F D S I P L L I N Y H Q H N G
 481 GGAGGCCTGGTGACTCGACTCCGGTATCCAGTTGTTGGAGGCAGAAAGCCCCAGTT 540
 G G L V T R L R Y P V C F G R Q K A P V
 541 ACAGCAGGGCTGAGATA CGGGAAATGGGTGATCGACCCCTCAGAGCTCACTTTGTGCAA 600
 T A G L R Y G K W V I D P S E L T F V Q
 601 GAGATTGGCAGTGGCAATTGGGTTGGTGCATCTGGCTACTGGCTAACAAAGGACAAG 660
 E I G S G Q F G L V H L G Y W L N K D K
 661 GTGGCTATCAAACCATTGGGAAGGGCTATGTCAGAAGAGGACTTCATAGAGGAGGCT 720
 V A I K T I R E G A M S E E D F I E E A

721 GAAGTAATGATGAAACTCTCTCATCCAACTGGTGCAGCTGTATGGGTGTGCCTGGAG 780
 E V M M K L S H P K L V Q L Y G V C L E

 781 CAGGCCCATCTGCCTGGTGTGAGTCATGGAGCACGGCTGCCTGTCAGATTATCTA 840
 Q A P I C L V F E F M E H G C L S D Y L

 841 CGCACCCAGCGGGACTTTGCTGCAGAGACCTGCTGGCATGTGTCAGATGTGT 900
 R T Q R G L F A A E T L L G M C L D V C

 901 GAGGGCATGGCCTACCTGGAAGAGGCATGTGTCATCCACAGAGACTTGGCTGCCAGAAAT 960
 E G M A Y L E E A C V I H R D L A A R N

 961 TGTTGGTGGGAGAAAACCAAGTCATCAAGGTGTCTGACTTTGGATGACAAGGTTGTT 1020
 C L V G E N Q V I K V S D F G M T R F V

 1021 CTGGATGATCAGTACACCAAGTCCACAGGCACCAAATTCCCGGTGAAGTGGCATCCCCA 1080
 L D D Q Y T S S T G T K F P V K W A S P

 1081 GAGGTTTCTTTCACTCGCTATAGCAGCAAGTCCGATGTGTCATTGGTGTGCTG 1140
 E V F S F S R Y S S K S D V W S F G V L

 1141 ATGTGGGAAGTTTCAGTGAAGGCAAAATCCCGTATGAAACCGAAGCAACTCAGAGGTG 1200
 M W E V F S E G K I P Y E N R S N S E V

 1201 GTGGAAGACATCAGTACCGGATTCGGTTGTACAAGCCCCGGCTGGCCTCCACACACGTC 1260
 V E D I S T G F R L Y K P R L A S T H V

 1261 TACCAGATTATGAATCACTGCTGGAAAGAGAGAGACCAGAAGATCGGCCAGCCTCTCCAGA 1320
 Y Q I M N H C W K E R P E D R P A F S R

 1321 CTGCTCGTCAACTGGCTGAAATTGCAGAATCAGGACTTAGGGATCC 1368
 L L R Q L A E I A E S G L *

FIG. 3 CONT'D

ITK MNNFILLEEOLIKKSQOKRRTSPSNFKVRFVLTKASLAYFEDRHGKKRTLKGSIELSRI 60
tITK ----- 0

PH Domain

ITK KCVETVKSDISIPCHYKYPFOVHDNYLLYVFAPDRESRQRWVLALKEETRNNNNSLVPKY 120
tITK ----- 0

ITK HPNFWMDGKWRCCSQLEKLATGCAQYDPTKNASKPLPPTPEDNRPLWEPEETVVIALY 180
tITK ----- MPEETVVIALY 11

SH3 Domain

ITK DYQTNDPQEELALRRNEEYCLDSSEIHWWRVQDENHGEGYVPSSYLVEKSPNNLETYEWY 240
tITK DYQTNDPQEELALRRNEEYCLDSSEIHWWRVQDRNGHEGYVPSSYLVEKSPNNLETYEWY 71

SH2 Domain

ITK NKSISRDKAEKLLLDTGKEGAFMVRDSRTAGTYTVSVFTKAVVSENNPCIKHYHIKETND 300
tITK NKSISRDKAEKLLLDTGKEGAFMVRDSRTAGTYTVSVFTKAVVSENNPCIKHYHIKETND 131

ITK NPKRYYVAEKYVFDSIPLLINYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYGKWIDP 360
tITK NPKRYYVAEKYVFDSIPLLINYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYGKWIDP 191

ITK SELTFVQEIGSGQFGLVLGYWLNKDKVAIKTIREGAMSEEDFIEEAEVMMKLSHPKLVQ 420
tITK SELTFVQEIGSGQFGLVLGYWLNKDKVAIKTIREGAMSEEDFIEEAEVMMKLSHPKLVQ 251

ITK LYGVCLEQAPICLVFEFMEHGCLSDYLRQGLFAAETLLGMCLDVCEGMAYLEEACVIH 480
tITK LYGVCLEQAPICLVFEFMEHGCLSDYLRQGLFAAETLLGMCLDVCEGMAYLEEACVIH 311

Kinase Domain

ITK RDLAARNCLVGENQVIKVSDFGMTRFVLDQYTSSTGTKFPVKWASPEVFSFSRYSSKD 540
tITK RDLAARNCLVGENQVIKVSDFGMTRFVLDQYTSSTGTKFPVKWASPEVFSFSRYSSKD 371

ITK VWSFGVLMWEVFSEGKIPYENRSNSEVVEDISTGFRLYKPRLASTHVYQIMNHCWKEPPE 600
tITK VWSFGVLMWEVFSEGKIPYENRSNSEVVEDISTGFRLYKPRLASTHVYQIMNHCWKERPE 431

ITK DRPAFSRLLROLAETAESGL 620
tITK DRPAFSRLLROLAETAESGL 451

FIG. 4

FIG. 5

		10	20	30	40	50	60	70	80	90
BTK_HUMAN	-MAA	VILLESI	FILKRSQQKKTSPLNFKRFLLT	VHKL	SYYEYDFERRRGSKKG	SIDVEKITC	ETVPEKNPPPERQIPRGEESSEM			
BTK_MOUSE	-AA	VILLESI	LKRSSQQKKTSPLNFKRFLLT	VHKL	SYYEYDFERRRGSKKG	SIDVEKITC	ETVPEKNPPPERQIPRGEESSEM			
ITK_HUMAN	MNIE	VILLE	QLIKKSQQKKRRTSPSNFKVRE	FLTKASLAYED	-RHGKKRTRLKGSIELSRIC	KCVELVKS				
ITK_MOUSE	MNIE	VILLE	QLIKKSQQKKRRTSPSNFKVRE	FLTKASLAYED	-RHGKKRTRLKGSIELSRIC	KCVELVKS				
TEC_HUMAN	INENT	VILLE	LIKRSQQKKTSP	FLNKYKERLEVFLTRSM	LTYYEG--RAEKY-RKGFI	DVKIRKCVELVKN				
TEC_MOUSE	MNEN	VILLE	LIKRSQQKKTSP	FLNKYKERLEVFLTRSM	LTYYEG--RAEKY-RKGFI	DVKIRKCVELVKN				
BMX_HUMAN	PDTKSI	ELLL	LIKRSQQKKTSP	FLNKYKERLEVFLTRSM	LTYYEG--DKMKGSRKG	SIEI	KIRKCVELVKN			
P97504	ME	SKS	ELLIKKSQQKKMSPNNY	FLNKYKERLEVFLTRSM	LTYYEG--DKMKGSRKG	SIEI	KIRKCVELVKN			
TXK_HUMAN										
TXK_MOUSE										
		100	110	120	130	140	150	160	170	180
BTK_HUMAN	EQIS	TIERFP	FQ-----VVYDEGPLYVESPTEELRKW	HLKVNIR	NSDLVQKYHPC	EWIDQYLCCSQTA	NAMGCQILENRNG			
BTK_MOUSE	EQIS	TIERFP	FQ-----VVYDEGPLYVESPTEELRKW	HLKVNIR	NSDLVQKYHPC	EWIDQYLCCSQTA	NAMGCQILENRNG			
ITK_HUMAN	-DIS	IPICHYKPFQ	-----VHDNYLILYFAPDRESQ	RWVLAKEE	TRINNSLVPKYH	PNEWMGDGRWRC	CQSLEKLATGCAOYDFT--			
ITK_MOUSE	-DIS	IPICHYKPFQ	-----VHDNYLILYFAPDRESQ	RWVLAKEE	TRINNSLVPKYH	PNEWMGDGRWRC	CQSLEKLATGCAOYDFT--			
TEC_HUMAN	DDGT	IPCPQNKFQ	-----VHDANTLYFAPSPQRDOLW	KKLKEE	TKINNNIMIKYH	PKETWTDSYQCC	RCOTEKLAPGCEKYNLIES			
TEC_MOUSE	DDGT	IPCPQNKFQ	-----VHDANTLYFAPSPQRDOLW	KKLKEE	TKINNNIMIKYH	PKETWTDSYQCC	RCOTEKLAPGCEKYNLIES			
BMX_HUMAN	LEEQT	PVERQPFQ	-----IVKDGLLYVYASNEE	RSQWLKALQKE	TKINPHLVKYHS	GTFVDGKFLCCQ	QOSCKAAPGCTLWEAYAN			
P97504	LEEQT	PVERQPFQ	-----IVKDGLLYVYASNEE	RSQWLKALQKE	TKINPHLVKYHS	GTFVDGKFLCCQ	QOSCKAAPGCTLWEAYAD			
TXK_HUMAN										
TXK_MOUSE										
		190	200	210	220	230	240	250	260	270
BTK_HUMAN	SLKP	GSSMRK	TKKPLPPTPEEQDILKKPLP	PEPAVSTSEL	KKVVALYDIMP	MANDLQLRKG	--DEYF			
BTK_MOUSE	SLKP	GSSMRK	TKKPLPPTPEEQDILKKPLP	PEPAVSTSEL	KKVVALYDIMP	MANDLQLRKG	--DEYF			
ITK_HUMAN	---	---	KNASKKPLPPTED--NRRPLW	---	EEETTVVIALYD	QTNDPQEELAIRRNN	--EEYC			
ITK_MOUSE	---	---	KNASKKPLPPTED--NRRPLW	---	EEETTVVIALYD	QTNDPQEELAIRRCD	--EEYC			
TEC_HUMAN	SI	---	TKALPPAPEI--KRRRPP	PPIEL--EEE	EEEDNSEIIVVAM	IDQAE	GMDLRLERG	--QEIYI		
TEC_MOUSE	SI	---	TKALPPAPEI--KRRRPP	PPIEL--EEE	EEEDNSEIIVVAM	IDQAE	GMDLRLERG	--QEIYI		
BMX_HUMAN	LHTAVNE	--	SHRV9TEPDR	--VLIKIPRAV	VLMKDAPS	SSSTLREQDNE	SKNYGSQPPSSSTS	LAQYDSNSK	YI	---
P97504	LHAIISDE	--	SHRA9TEPDR	--VLIKIPRAV	VLMKDAPS	SSSTLREQDNE	SKNYGSQPPSSSTS	LAQYDSNSK	YI	---
TXK_HUMAN	QSNNTGRV	QPSK	RKPLPPLPP	--	SEVAEKKIQV	KALYDFLPREP	CNLALLRA	--EEYL		
TXK_MOUSE	QSNRG	GGVQSP	SKRKPLPPLPP	--	QEPPD	ERIQV	KALYDFLPREP	CNLALLRA	--EEYL	

BTK_HUMAN	<u>I</u> LEESNL ^P W ^R ARDKNGQ---EGYIPSN-	280	290	300	310	320	330	340	350	360
BTK_MOUSE	<u>I</u> LEESNL ^P W ^R ARDKNGQ---EGYIPSN-									
TK_HUMAN	LLDSSEIMMWTRVQDRNGH---EGYIPSS-									
TK_MOUSE	LLDSSEIMMWTRVQDKNGH---EGYIPSS-									
TEC_HUMAN	ILKEKNDLHW ^R ARDKYG---EGYIPSN-									
TEC_MOUSE	ILKEKNDLHW ^R ARDKYG---EGYIPSN-									
BMX_HUMAN	IPREFDPDWQVRKLKSSSSEDVAASSNQKERNVN-HTTSKISWEFPESSSEEENLDYDWEAGNISRSQSEQLLRQKGKEGAENVRN P97504									
TK_HUMAN	ILKEKYNPHW ^R KARDRGN---EGLIPSN-									
TK_MOUSE	ILERCDPMW ^R KARDRGN---EGLIPSN-									
BTK_HUMAN	SSEAGCKYT ^S VEAKSTGDPQ-GVIRHYVVCSTIPQS--QYYLAEKHLFSTIPELINYHQHNSAGLISRLKYPVSQQQNKNAPSTAGLGYSW	370	380	390	400	410	420	430	440	450
BTK_MOUSE	SSEAGCKYT ^S VEAKSTGEPQ-GVIRHYVVCSTIPQS--QYYLAEKHLFSTIPELINYHQHNSAGLISRLKYPVSQQQNKNAPSTAGLGYSW									
TK_HUMAN	SRTAGTYTVS ^T VETKAVVSENNPCKHYHKETENDNPCKRYVVAEKYVFSIPLIINYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYGKW									
TK_MOUSE	SRTAGTYTVS ^T VETKAVVSENNPCKHYHKETENDNPCKRYVVAEKYVFSIPLIINYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYGKW									
TEC_HUMAN	SSQPGGLITVSLTKEGGEGS-SGFRHYHIKETTSPKKKYLAEKHAEGSIPELIELYHKHNAAGLVLTRLRYPVSTRGKNAPTTAGFSYDKW									
TEC_MOUSE	SSQPGGLITVSLTKEGGEGS-SGFRHYHIKETTSPKKKYLAEKHAEGSIPELIELYHKHNAAGLVLTRLRYPVSTRGKNAPTTAGFSYDKW									
BMX_HUMAN	SSQVGCMZTVSLESKAVNDKK-GTVKHYHVHTNAEN--KLYLAENYCDFSIPKLIYHQHNSAGMITSRLRPVSTKANKVPDSVSIGNITW P97504									
TK_HUMAN	SSQMGMYTVSLESKAVNDKK-GTVKHYHVHTNAEN--KLYLAENYCDFSIPKLIYHQHNSAGMITSRLRPVSTKANKVPVSVALGSGIW									
TK_MOUSE	SRHLSYTISVEMGARRSTE-AAIKHYQIKKNDSG--QWYVAERHAFQSIPELIWIYHQHNAAGLMTRLRYPVGLMGSCLPATAGESYEKW									
TK_MOUSE	SRHLSYTISVETARRHTQ-SSIKHYQIKKNDSG--QWYITERHLEPSVPELIQHQYNAAGLTSRLRYPPIGLGSCLPATSGESYEKW									

FIG. 5 CONT'D

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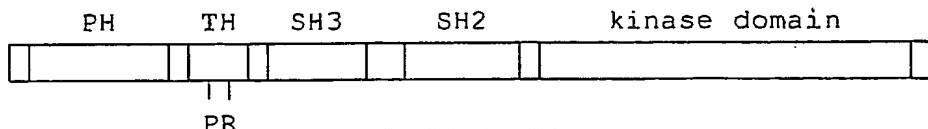


FIG. 6

3 prime Oligo: GATCGGATCCGAAGCTTATTGGCGAGCTCAGGATTC

5 prime Oligo: GATCGCGGCCGCACCATGGCAGCAGCACCAAGTCTCCACAAGTG

FIG. 7

MAAAPVSTSELKKVVALYDYMPMNANDLQLRGDE
YFILEESNLPPWRARDKNGQEGYIPSNYVTEAEDSIEMYEWYSKHMTRSQAELLKQEGK
EGGFIVRDSSKAGKYTVSVFAKSTGDPQGVIRHYVVCSTPQSQYYLAEKHLFSTIPELIN
YHQHNSAGLISRALKYPVSQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQFGVVKYGK
WRGQYDVAIKMIKEGSMSEDEFIEEAKVMMNLSHEKLVQLYGVCTKQRPIIFIITEYMANG
CLLNLYLREMRFQQTQQLLEMCKDVCEAMEYLESKQFLHRDLAARNCLVNDQGVVKVSDF
GLSRYVLDDEYTSSVGSKFPVRWSPPPEVIMYSKFSSKSDIWAFGVLMWEIYSLGKMPYER
FTNSETAEHIAQGLRLYFPHLASEKVYTIMYSCWHEKADERPTFKILLSNI LDVMDEES

FIG. 8

SUBSTITUTE SHEET (RULE 26)

ATGGCAGCAGCACCAAGTCTCCACAAGTGAGCTG
AAAAAGGTTGTGCCCTTATGATTACATGCCAATGAATGCAAATGATCTACAGCTGC
AAGGGTGATGAATATTTATCTTGAGGAAAGCAACTTACCATGGTGGAGAGCACGAGAT
AAAATGGGCAGGAAGGCTACATTCTAGTAACATATGTCAGTGAAGCGGAAGACTCCATA
GAAATGTATGAGTGGTATTCCAAACACATGACTCGGAGTCAGGCTGAGCAACTGCTAAAG
CAAGAGGGAAAGAAGGAGGTTCATGTCAGAGACTCCAGCAAAGCTGGCAAATATACA
GTGTCTGTGTTGCTAAATCCACAGGGGACCCTCAAGGGGTGATACTGTCATTATGTTGTG
TGTTCCACACCTCAGAGCCAGTATTACCTGGCTGAGAAGCACCTTTCAGCACCATCCCT
GAGCTCATTAACTACCACAGCACAACTCTGCAGGACTCATATCCAGGCTCAAATATCCA
GTGTCTCAACAAAACAAGAATGCACCTTCCACTGCAGGCCTGGATACGGATCATGGAA
ATTGATCCAAAGGACCTGACCTTCTTGAAGGAGCTGGGACTGGACAATTGGGGTAGTG
AAGTATGGAAATGGAGAGGCCAGTACGACGTGGCCATCAAGATGATCAAAGAAGGCTCC
ATGTCTGAAGATGAATTCAATTGAAGAACGCAAAGTCATGATGAATCTTCCCCTGAGAAG
CTGGTGCAGTTGTATGGCTCTGCACCAAGCAGCGCCCCATCTCATCATCACTGAGTAC
ATGGCAATGGCTGCCTCCTGAACCTACCTGAGGGAGATGCGCCACCGCTCCAGACTCAG
CAGCTGCTAGAGATGTGCAAGGATGTCTGTGAAGCCATGGAATACCTGGAGTCAAAGCAG
TTCCCTCACCGAGACCTGGCAGCTCGAAACTGTTGGTAAACGATCAAGGAGTTGTTAAA
GTATCTGATTGCGCTGTCCAGGTATGTCCTGGATGATGAATACACAAGCTCAGTAGGC
TCCAAATTCCAGTCCGGTGGTCCCCACCGGAAGTCTGATGTATAGCAAGTTCAGCAGC
AAATCTGACATTGGCTTTGGGTTTGATGTTGGAAATTACTCCCTGGGAAGATG
CCATATGAGAGATTTACTAACAGTGAGACTGCTGAACACATTGCCAAGGCCTACGTCTC
TACAGGCCTCATCTGGCTTCAGAGAAGGTATATACCATCATGTACAGTTGCTGGCATGAG
AAAGCAGATGAGCGTCCACTTCAAAATTCTCTGAGCAATATTCTAGATGTCATGGAT
GAAGAATCCTGA

FIG. 9

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A61K 38/45

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(71) Applicant (for all designated States except US): GLAXO
GROUP LIMITED [GB/GB]; Glaxo Wellcome House,
Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SIMS, Martin, John
[GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Steve-
nage, Hertfordshire SG1 2NY (GB). HAYES, David, John
[GB/GB]; GlaxoSmithKline, Gunnels Wood Road, Steve-
nage, Hertfordshire, SG1 2NY (GB).

(74) Agent: CRAWLEY, Karen; GlaxoSmithKline, Corporate
Intellectual Property (CN9.25.1), 980 Great West Road,
Brentford, Middlesex TW8 9GS (GB).

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GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI,
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patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
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CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



A3

02/034899 A3

(54) Title: TEC KINASE ASSAY

WO

(57) Abstract: This invention relates to truncated Tec kinase polypeptides and their use in screening for compounds which modulate the activity of Tec kinase polypeptides. Also described are nucleotide sequences encoding truncated Tec kinase polypeptides, vectors and host cells containing said nucleotides.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/11949

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/12	C12N15/54	C12N5/10	G01N33/573	A61P29/00
C12Q1/48	C12Q1/68	A61K38/45		

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MA YONG-CHAO ET AL: "Identification of the binding site for Gqalpha on its effector Bruton's tyrosine kinase." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 21, 13 October 1998 (1998-10-13), pages 12197-12201, XP002213941 Oct. 13, 1998 ISSN: 0027-8424 figures 1B,4 page 12201, paragraph 2	1,4,7-9, 12-19
Y	---	5,6,10, 11 -/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

25 September 2002

10/10/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Aslund, J

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/11949

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>AUGUST AVERY ET AL: "Src-induced activation of inducible T cell kinase (ITK) requires phosphatidylinositol 3-kinase activity and the Pleckstrin homology domain of inducible T cell kinase."</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 94, no. 21, 1997, pages 11227-11232, XP002213942</p> <p>1997</p> <p>ISSN: 0027-8424</p> <p>figure 1</p> <p>---</p>	5, 6, 10, 11
X	<p>VASSILEV ALEXEI ET AL: "Bruton's tyrosine kinase as an inhibitor of the Fas/CD95 death-inducing signaling complex."</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 3, 15 January 1999 (1999-01-15), pages 1646-1656, XP002213939</p> <p>ISSN: 0021-9258</p> <p>figures 4,,8A</p> <p>---</p>	1, 4, 7-9, 12-17
X	<p>HULKOWER KEREN I ET AL: "Induction of prostaglandin H synthase-2 and tumor necrosis factor-alpha in human amniotic WISH cells by various stimuli occurs through distinct intracellular mechanisms."</p> <p>JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, vol. 280, no. 2, 1997, pages 1065-1074, XP002214226</p> <p>ISSN: 0022-3565</p> <p>figure 8; table 1</p> <p>---</p>	21-26
X	<p>AGULLO GEORGINE ET AL: "Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: A comparison with tyrosine kinase and protein kinase C inhibition."</p> <p>BIOCHEMICAL PHARMACOLOGY, vol. 53, no. 11, 1997, pages 1649-1657, XP002214227</p> <p>ISSN: 0006-2952</p> <p>the whole document</p> <p>---</p> <p>-/-</p>	21-26

INTERNATIONAL SEARCH REPORT

I	tional Application No PCT/EP 01/11949
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WALKER EDWARD H ET AL: "Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine." MOLECULAR CELL, vol. 6, no. 4, October 2000 (2000-10), pages 909-919, XP002214228 ISSN: 1097-2765 the whole document</p> <p>---</p>	21-26
A	<p>SHIRAI T ET AL: "Specific detection of phosphatidylinositol 3,4,5-trisphosphate binding proteins by the PIP3 analogue beads: An application for rapid purification of the PIP3 binding proteins" BIOCHIMICA ET BIOPHYSICA ACTA. MOLECULAR CELL RESEARCH, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 1402, no. 3, 24 April 1998 (1998-04-24), pages 292-302, XP004277780 ISSN: 0167-4889 page 301, column 2, paragraph 1; figure 7</p> <p>---</p>	1-26
A	<p>CICCARELLI FRANCESCA D ET AL: "Large and diverse numbers of human diseases with HCK mutations." HUMAN MOLECULAR GENETICS, vol. 9, no. 6 Spec. Review Issue, 12 April 2000 (2000-04-12), pages 1001-1007, XP002213878 ISSN: 0964-6906 page 1004, column 2, paragraph 2 -page 1005, column 1, paragraph 1</p> <p>---</p>	1-26
A	<p>KAWAKAMI YUKO ET AL: "Tec family protein-tyrosine kinases and pleckstrin homology domains in mast cells." IMMUNOLOGY LETTERS, vol. 54, no. 2-3, 1996, pages 113-117, XP002213940 ISSN: 0165-2478 page 116</p> <p>---</p>	1-26
A	<p>YANG WEN-CHIN ET AL: "Tec kinases: A family with multiple roles in immunity." IMMUNITY, vol. 12, no. 4, April 2000 (2000-04), pages 373-382, XP002213879 ISSN: 1074-7613</p> <p>---</p>	
	-/--	

INTERNATIONAL SEARCH REPORT

I tional Application No
PCT/EP 01/11949

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GIBSON S ET AL: "IDENTIFICATION, CLONING, AND CHARACTERIZATION OF A NOVEL HUMAN T-CELL-SPECIFIC TYROSINE KINASE LOCATED AT THE HEMATOPOIETIN COMPLEX ON CHROMOSOME 5Q"</p> <p>BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 82, no. 5, 1 September 1993 (1993-09-01), pages 1561-1572, XP000611524</p> <p>ISSN: 0006-4971</p> <p>---</p>	
A	<p>ANDREOTTI AMY HAMILTON ET AL: "Regulatory intramolecular association in a tyrosine kinase of the Tec family."</p> <p>NATURE (LONDON), vol. 385, no. 6611, 1997, pages 93-97, XP002214229</p> <p>ISSN: 0028-0836</p> <p>---</p>	
A	<p>MANO HIROYUKI: "Tec family of protein-tyrosine kinases: An overview of their structure and function."</p> <p>CYTOKINE & GROWTH FACTOR REVIEWS, vol. 10, no. 3-4, 1999, pages 267-280, XP001113173</p> <p>ISSN: 1359-6101</p> <p>---</p>	
A	<p>LI ET AL.: "Activation of Bruton's tyrosine kinase (BTK) by a point mutation in its pleckstrin homology (PH) domain."</p> <p>IMMUNITY, vol. 2, no. 5, May 1995 (1995-05), pages 451-460, XP008008518</p> <p>ISSN: 1074-7613</p> <p>---</p>	
T	<p>HAO SHENGLI ET AL: "The proline rich region of the Tec homology domain of ITK regulates its activity."</p> <p>FEBS LETTERS, vol. 525, no. 1-3, 2002, pages 53-58, XP004375894</p> <p>14 August, 2002</p> <p>ISSN: 0014-5793</p> <p>page 54</p> <p>---</p> <p>-/-</p>	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/11949

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	LOWRY WILLIAM E ET AL: "Role of the PHTH module in protein substrate recognition by Bruton's agammaglobulinemia tyrosine kinase." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 48, 30 November 2001 (2001-11-30), pages 45276-45281, XP002214230 November 30, 2001 ISSN: 0021-9258 the whole document -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/11949

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 25,26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: 21-26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 21-26

Present claims 21-26 relate to a compound defined by reference to a desirable characteristic or property, namely the ability to modulate TEC kinase activity. The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to staurosporin and the flavone compounds described in Table 1 (page 22).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.